

REMARKS

Status Summary

Claims 28, 29, 34–36, 41, 43, and 52 are pending. Claims 1–27, 30–33, and 37–40 were canceled previously. Claims 42, 44–51, and 53–59 were previously withdrawn. Claims 28, 29, 34–36, 41, 43 and 52 are rejected under 35 U.S.C. § 112, second paragraph, as allegedly indefinite. Claims 28, 29, 34–36, 41, 43 and 52 are rejected under 35 U.S.C. § 112, first paragraph as allegedly failing to comply with the enablement requirement. Reconsideration in view of foregoing amendments and the following remarks is respectfully requested.

Request for Interview with Examiner

Should the Examiner not find the claim amendments and remarks herein persuasive to overcome all outstanding rejections and place the claims in condition for allowance, an interview with the Examiner is respectfully requested to discuss the outstanding rejections.

Discussion of Amendments

Amendments to claims 28, 29, 34, and 35 are supported by at least paragraphs [0018], [0019], [0021], [0083], [0099], [0175]–[0181], [0360], [0372], [0384], [0391], [0402], [0458], [0460], [0461], [0469]–[0471], [0581], and [0593], inter alia. No new matter is added by way of these amendments and the claims will be placed in condition for allowance or in better condition for consideration upon appeal.

Rejections Under 35 U.S.C. §112, Second Paragraph

Claims 28, 29, 34–36, 41, 43 and 52 are currently rejected under 35 U.S.C. § 112, second paragraph, as allegedly indefinite. Specifically, the Examiner alleges that one of ordinary skill in the art could not reasonably determine the metes and bounds of the claims. *Official Action*, at 2–3.

In reviewing a claim for compliance with 35 U.S.C. § 112, second paragraph, the examiner must consider the claim as a whole to determine whether the claim apprises one of ordinary skill in the art of its scope and, therefore, serves the notice function required by 35 U.S.C. § 112, second paragraph, by providing clear warning to others as to what constitutes

infringement of the patent. *See, e.g., Solomon v. Kimberly-Clark Corp.*, 216 F.3d 1372, 1379, 55 U.S.P.Q.2d 1279, 1283 (Fed. Cir. 2000).

Independent claims 28 and 35 are directed to tobacco plants and to substantively conform them to the language used in claim 1 of U.S. Patent 5,939,602 (“the ‘602 patent”); issued August 17, 1999; corresponding to U.S. Application 08/808,931, filed Dec. 20, 1997; examined by Amy J. Nelson in Art unit 1649), which is a great, great, great, great grandparent of the current application (through continuations and continuations-in-part) and which the instant application claims priority. Claim 1 of the ‘602 patent is:

1. A modified plant DNA molecule encoding a modified enzyme having protoporphyrinogen oxidase activity, wherein said modified enzyme has at least one amino acid modification compared to a naturally occurring protox enzyme, wherein said at least one amino acid modification confers resistance to an inhibitor of the naturally occurring protox enzyme, and wherein said at least one amino acid modification comprises an amino acid substitution occurring at a position corresponding to position 240, 245, 246, 388, 390, 451, 455, 500, or 536 of the comparative alignment shown in Table 1.

U.S. Patent No. 5,939,602 col. 145, ll. 30–40 (filed Dec. 20, 1997). Table 1 of the instant application is also very similar to Table 1 of the ‘602 patent. Because an issued patent has a statutory presumption of validity, the amended claims conforming to those of the ‘602 patent referring to an amino acid modification corresponding to a position in a sequence in Table 1 are also not indefinite.

It is further respectfully asserted that one having ordinary skill in the art is clearly apprised of the scope of the claims because one having ordinary skill in the art would understand the metes and bounds of a DNA sequence encoding a protox enzyme “wherein [] at least one amino acid modification comprises an amino acid substitution occurring at a position corresponding to position 221, 226, 227, 369, 371, 432, 436, 481, or 517 of SEQ ID NO: 12 in the comparative alignment shown in Table 1.” Table 1 expressly identifies specific examples of

plant protox enzymes that fall within the scope of the claims (Table 1A has been reproduced herein in **Figure 1** in Appendix A, *infra*). Further Table 1A of the specification shows amino acids at positions 221, 226, 227, 369, 371, 432, 436, 481, or 517 of SEQ ID NO: 12 in bold font, so that one having ordinary skill in the art could easily identify the amino acids in any sequence in question that correspond to these demarked positions.

As noted above, the Examiner alleges that one of ordinary skill in the art could not reasonably determine the metes and bounds of the claim. *Official Action*, at 2–3. As will be shown below, one having ordinary skill in the art can indeed reasonably determine the scope of the claimed invention.

First, currently amended claim 28 specifies a method for expressing a modified enzyme in a tobacco plant plastid. This preamble conveys that the claim is a process claim and that the context of the method is to express a modified enzyme (*i.e.*, a protein) in a plant organelle. The method comprises introducing a chimeric gene into a tobacco plant plastid genome (*i.e.*, the plastome).

The steps of this method are to use a modified DNA molecule that encodes for a modified enzyme (*i.e.*, an enzyme that has been designed or engineered) with protoporphyrinogen oxidase (protox) activity that is normally targeted to a plant plastid by a plastid transit peptide. In other words, the DNA encodes a protox enzyme lacking the plastid-targeting signal peptide. The DNA encoding the protox enzyme is modified by deleting the coding sequence for the plastid transit peptide, so that the expressed enzyme will not be targeted to the plastid, but will remain in the cytoplasm. Further, the enzyme has at least one amino acid modification compared to the naturally occurring protox enzyme. This indicates that the protox enzyme must contain at least one site-directed modification to an amino acid. The following clauses specify the effect(s) of the modification(s) and the position(s) of the modification(s). The effect(s) of the modification(s) is (are) to confer resistance to a protox inhibitor of the naturally occurring protox enzyme. In other words, the modifications make the protox enzyme resistant to the inhibitor, namely a herbicide.

In addition, at least one modification to the protox enzyme is an amino acid substitution occurring at a position corresponding to position 221, 226, 227, 369, 371, 432, 436, 481, or 517 of SEQ ID NO: 12 in the comparative alignment shown in Table 1. This clause troubles the Examiner because he alleges that it is “unclear” and that it “does not define any particular sequence that would be used” for expressing the enzyme.

Applicant’s position is that one having ordinary skill in the art can clearly determine the scope of the claimed invention. As the Examiner alleges, the claim covers DNA sequences encoding protox enzymes. The claim has been amended to cover only plant protox enzymes, so this defines the claim scope. Moreover, the claim states that the DNA and the encoded protein are *modified*, so this excludes naturally occurring sequences. Naturally occurring protox genes can be used as a starting-point for DNA sequences of the present invention, but modifications must be made to the sequences before they come under the scope of the claims. Further, the modifications must make the protox enzyme (*i.e.* the protein product) resistant to an inhibitor herbicide.

The claimed protox enzyme is inherently functionally active and resistant to the inhibitor. This further defines the scope of DNA sequences to include only those that can encode *functional* enzymes that are *resistant* to inhibitors. Furthermore, the claim scope is defined by explicitly specifying that at least one amino acid modification must occur at a position corresponding to position 221, 226, 227, 369, 371, 432, 436, 481, or 517 of SEQ ID NO: 12 in the comparative alignment shown in Table 1. See **Figure 1**, *infra* in Appendix A. Collectively, the ambit of sequences that meet all of the foregoing is finite. The sequence must encode a protox enzyme that has been modified, that is resistant to an inhibitor, and that at least one of the modifications occurs at a position corresponding to position 221, 226, 227, 369, 371, 432, 436, 481, or 517 of SEQ ID NO: 12 in the comparative alignment shown in Table 1. Only a defined number of DNA sequences will fulfill these criteria. A BLAST search using SEQ ID NO: 11 (DNA sequence encoding the *Glycine max* protox enzyme in SEQ ID NO: 12) produced nine (9) substantive hits (performed Nov. 19, 2010). A BLAST search using SEQ ID NO: 12 (protein sequence of *Glycine max* protox enzyme) produced thirty-eight (38) hits with 50% or greater

sequence identity. Based on these results, the number of potential natural sequences that could be modified to be covered by the presently claim invention is not indefinite.

The Examiner also appears perplexed by the claim phrase “corresponding to” in the clause “at least one amino acid modification must occur at a position *corresponding to* position 221, 226, 227, 369, 371, 432, 436, 481, or 517 of SEQ ID NO: 12 in the comparative alignment shown in Table 1.” Claims must be given their broadest reasonable interpretation consistent with the specification. *Phillips v. AWH Corp.*, 415 F.3d 1303, 75 U.S.P.Q.2d 1321 (Fed. Cir. 2005). The term “corresponding” must be given its plain meaning unless it inconsistent with the specification. *In re Zletz*, 893 F.2d 319, 321, 13 U.S.P.Q.2d 1320, 1322 (Fed. Cir. 1989). Further, the phrase “corresponding to” is expressly defined in the specification. See Appendix B, which contains a reproduction of page 5 of the published application. Paragraph 83 of the specification defines “corresponding to”:

[0083] Corresponding To: in the context of the present invention, “corresponding to” means that when the amino acid sequences of various protox enzymes are aligned with each other, such as in Table 1A, the amino acids that “correspond to” certain enumerated positions in Table 1A are those that align with these positions in Table 1A, but that are not necessarily in these exact numerical positions relative to the particular protox enzyme’s amino acid sequence. Likewise, when the amino acid sequence of a particular protox enzyme (for example, the soybean protox enzyme) is aligned with the amino acid sequence of a reference protox enzyme (for example, the *Arabidopsis* protox-1 sequence given in SEQ ID NO:2), the amino acids in the soybean protox sequence that “correspond to” certain enumerated positions of SEQ ID NO:2 are those that align with these positions of SEQ ID NO:2, but are not necessarily in these exact numerical positions of the soybean protox enzyme’s amino acid sequence.

Moreover, the dictionary definition of “*corresponding*” is “having or participating in the same relationship (as kind, degree, *position*, correspondence, or function) *especially with regard to the same or like wholes . . .*” *Corresponding Definition*, Merriam-Webster Online Dictionary,

<http://www.merriam-webster.com/dictionary/corresponding> (last visited Nov. 19, 2010) emphasis added. Synonyms for “*corresponding*” include “akin, analogous, cognate, comparable, connate, correspondent, alike, ditto, like, matching, parallel, resemblant, resembling, similar, such, suchlike” *Id.* Accordingly, this claim term indicates that one should take a protox amino acid sequence (translated from the encoding modified DNA sequence that is the subject of the claim) and align its sequence with the comparative alignment shown in Table 1A of the specification. Upon aligning the sequence, one having ordinary skill in the art will notice that particular amino acids will “*correspond to*,” *i.e.*, be identical to, similar to, analogous to, or merely occupy the same ordinal position as the amino acids of SEQ ID NO: 12 in the comparative alignment. *See Figure 1, infra* in Appendix A. Once this relationship is established, one can easily determine the residues of the sequence in question that “*correspond to*,” *i.e.*, are identical to, similar to, analogous to, or merely occupy the same ordinal position in the alignment as the amino acids at positions 221, 226, 227, 369, 371, 432, 436, 481, or 517 of SEQ ID NO: 12 in the comparative alignment. Moreover, the amino acid residues at positions 221, 226, 227, 369, 371, 432, 436, 481, or 517 of *all* proteins in the alignment are indicated in bold font to emphasize these positions. In other words, the amino acids of the sequence in question, once aligned, will unequivocally *correspond to* a position in SEQ ID NO: 12 in the comparative alignment that are in bold font. Consequently, those *corresponding* amino acids must be modified to produce a protox enzyme resistant to an inhibitor to come within the scope of the claims. One having ordinary skill in the art would have no trouble understanding what DNA sequences and the correspondingly encoded proteins are covered by these claims.

The Examiner further proposes that claim 29’s alleged indefiniteness is exemplified by “a DNA sequence with 95% similarity, but [having] a frame deletion at position 100, and also [having] a mutation at position 222.”

First, the Examiner’s example *is* indefinite, because it does not provide a frame of reference for numbering the DNA sequence. Does the numbering begin in the UTR or at the AUG codon? The presently claimed invention makes perfectly clear that the numbering frame is that of SEQ ID NO: 12 in Table 1, *i.e.*, the protein sequence, NOT the DNA sequence. *See Figure 1, infra* in Appendix A. This claim term is critical because it excludes all DNA

sequences that do not encode a functional, modified protox enzyme that is resistant to an inhibitor. For the sake of example, a DNA sequence with 95% identity to SEQ ID NO: 11 (the DNA encoding SEQ ID NO: 12) that has a frameshift at position 100 and a mutation at position 222 from the start codon, respectively, can easily be constructed. The DNA sequence encoding the protein of SEQ ID NO: 12 contains 1631 nucleotides (including the TAG amber stop codon). To impart 95% identity, 81 nucleotides were randomly mutated using the Mutate DNA application at the Sequence Manipulation Suite, <http://www.bioinformatics.org/sms2/index.html> (last visited Nov. 19, 2010). In addition, nucleotide 100 was deleted (Δ A100), creating a frame shift, and C222 was mutated to G (C222G), creating a proline-to-alanine mutation in the protein. This sequence was then translated and produced the following amino acid sequence:

```
>SEQ ID NO:11 95%ID  $\Delta$ A100 C222G
MVSVFNEILFPSPQTLRPSLHSPSTTFTSPTRNSLALTLTLCAAPLRNRPRLPKPE
TPPPWTESS*AEA*AASASPRPSPLNTPMPTSSSELTPEIASAATSPRWRTDTSKKKPT
ASSLLTQCSFWWTVV*RMSLFWGILMHLGLCCETGS*GRCPGS*LICLSLT**ALVAKS
GLALVRLEFGLLHQVMNRNLKRFLVDTLVMRFLNG**SLFVQGSMTIL*N*V*KQHSKG
FGSWKKMLVALLVER*KQYKREMLQNHLENRVCCNQVRMLDLSGRYLLCCIMQFLPD*
ATK*SYLGSFQVLVSWIVETTV*HMKHQKEWFLCSAKLLS*PFLPMLLVYCCVLCCLLLQ
MHFQSFITLQLQYPYPIQKLLDQNA**MVS*RGMVNCIHVVKEWKH*ELYTAHSSPT
EHHLEGFES*ITLEEQLILEFYRRRTVNWLKQLIEI*GKSL*QMPRTQL*WG*DCGLTL
FHSS*LAILIF*MLLKLSEILGLKGC SLGLIMCLVLPWDALRVFMR*QLK*TI FTQIE
CIN
```

This sequence was then aligned to the comparative alignment by sight (*i.e.*, not using an alignment program). As can be seen in the NTAA100C222G field of **Figure 1** (Appendix A), it is simple to align the translated sequence with SEQ ID NO: 12 (only the initial 69 amino acids were aligned on the alignment because of the stop codon at position 70). However, as could have been easily predicted by one having ordinary skill in the art, a deletion causing a frame shift at position 100 alters the reading frame after amino acid 33, thus producing a nonsense protein product that terminates at position 69. This protein would clearly not fall within the scope of the claims because: (i) it does not produce a functional, modified protox enzyme; (ii) no amino acid modification confers resistance to an inhibitor of the naturally occurring protox enzyme; and (iii) none of the amino acid modifications comprise a substitution occurring at a position corresponding to position 221, 226, 227, 369, 371, 432, 436, 481, or 517 of SEQ ID NO: 12 in the comparative alignment shown in Table 1 (*and see Figure 1* in Appendix A). Moreover, one having ordinary skill in the art would recognize that any DNA sequence with frame shifts,

deletions, or mutations that introduce premature stop codons would not produce a viable protox enzyme of the claimed invention, regardless of the modifications.

Notwithstanding, a realistic example is worth demonstrating. If one takes the Examiner's criteria and applies it to a protein sequence as the frame of reference as is indicated in the claims, it is clear that the currently amended claimed invention is not indefinite. The sequence in SEQ ID NO: 12 contains 543 amino acids. A sequence with 95% identity to SEQ ID NO: 12 would have about 28 mutations; these mutations were randomly introduced using the Mutate Protein application at the Sequence Manipulation Suite, <http://www.bioinformatics.org/sms2/index.html> (last visited Nov. 19, 2010). In addition, arginine 100 (Δ R100) was deleted and amino acid 222 was changed from serine to leucine (S222L). Furthermore, amino acid 221 was mutated to an alanine (C221A). The resulting sequence follows. The deletion and random mutations are indicated by underlining; the C221A and S222L mutations are bolded:

```
>SEQ ID NO:12 95%ID  $\Delta$ R100 S222L
MVSVFNEILFFPNQTLRLPSLHSPSTISFTSPTRKFPRSRPNPILRCSIAEESHASPFKTR
TSAPVDCVVVGGSVSGKHQAALATKNANANVVVTEARD_VGGNITTMERDGLWEMPPNS
FQSPDMLTMVVDSSGLKQELVLGDPDAPRFVLWNRKLRPKPGKLTDLPPFFDLMSIGGKIR
AGFGAEGIRPPPPGHEESVTEFVRRNLGDEVFERLIEPFALGVYAGDPSKLSMKAAFGKV
WKLEKNGGSIIGGTFKAIQERNGASKPPRPDRLPLPKPGQTVGSRFKGLTMLPD AISARLG
NKVKLSWKLSSISKLDSGEYSLTYETPSGVVSLQCKTVVLTIP EYVASTLLRPLSAAIAD
ALSTFYYPVAAVSIQYPKEWIRSEKLIDGELKGFQQLHPRSQGVETLTGIYSSSLF $\Delta$ NR
APPGRVLLNPIGGAMNTGILSKTDSSELVETVDRDLRKILINPNAQDPFVVGVRLWPQAI
PQFLVGHLDLLDVAKISIRNTGFEGFLGNGYVSGVALGRCVEGAYEAAEVNDFLTNRV
YK*
```

This sequence was then aligned to the comparative alignment by sight (*i.e.*, not using an alignment program). As can be seen in the AAAR100S222L field of **Figure 1**, in Appendix A, the sequence can easily be aligned with SEQ ID NO: 12 despite the deletion at position 100, the mutation at position 222, and the introduction of 28 other random mutations (shown as underlined residues). All that was required to align the sequence was to introduce a gap at position 100 corresponding to the deletion of arginine 100. Furthermore, the particular amino acid residues that correspond to positions 221, 226, 227, 369, 371, 432, 436, 481, or 517 of SEQ ID NO: 12 in the alignment are easily identified (highlighted and indicated with asterisks in **Figure 1** in Appendix A). Accordingly, the DNA encoding this protein would fall within the scope of the claimed invention because: (i) it is a modified DNA molecule encoding a modified enzyme having protoporphyrinogen oxidase activity . . . , (ii) the modified enzyme has at least

one at least one amino acid modification compared to a naturally occurring protox enzyme; (iii) the amino acid modification confers resistance to protox inhibitor; and (iv) the modification comprises an amino acid substitution occurring at a position corresponding to position 221 of SEQ ID NO: 12 in the comparative alignment shown in Table 1. In this particular case, there has been modification of the amino acid corresponding to amino acid 221 of SEQ ID NO: 12 (*i.e.*, C220A of the mutant protein) which will confer resistance to a protox inhibitor.

Based on this example, one having ordinary skill in the art could easily reverse transcribe this protein sequence to produce a modified DNA molecule encoding a modified enzyme having protox activity that is normally targeted to a plant plastid by a plastid transit peptide, where the modified DNA molecule is modified such that a coding sequence of the plastid transit peptide is absent from the modified DNA molecule, where the modified enzyme has at least one amino acid modification compared to a naturally occurring protox enzyme, where the at least one amino acid modification confers resistance to an inhibitor of the naturally occurring protox enzyme, and wherein the at least one amino acid modification comprises an amino acid substitution occurring at a position corresponding to position 221, 226, 227, 369, 371, 432, 436, 481, or 517 of SEQ ID NO: 12 in the comparative alignment shown in Table 1 of the specification.

Without being bound to any particular theory, but as envisioned, an artisan having ordinary skill in the art would typically begin with a plant DNA sequence encoding a function protox enzyme and then make amino acid modifications that would correspond to positions 221, 226, 227, 369, 371, 432, 436, 481, or 517 of SEQ ID NO: 12 in the comparative alignment in Table 1.

Collectively, the Examiner's allegations of indefiniteness for claim 28 are now traversed because the currently amended claims clearly apprise one having ordinary skill in the art of what the applicants claim as their invention. Currently amended claim 28 is an independent claim and claims 29 and 34 depend therefrom. Consequently, because currently amended claim 28 is allowable, claims 29 and 34 are also allowable. Furthermore, because currently amended claim 35 uses practically identical language to currently amended claim 28, currently amended claim 35 is now not indefinite for the same reasons that currently amended claim 28 is not indefinite.

Currently amended claim 35 is an independent claim and claims 36, 41, 43, and 52 depend therefrom. Accordingly, because currently amended claim 35 is allowable, 6, 41, 43, and 52 are also allowable.

Consequently, the rejections under 35 U.S.C. § 112, second paragraph have been traversed based on the current amendments and remarks. Withdrawal of the rejections of claims 28, 29, 34–36, 41, 43, and 52 under 35 U.S.C. § 112, second paragraph is respectfully requested.

Rejections Under 35 U.S.C. § 112, First Paragraph

Claims 28, 29, 34–36, 41, 43, and 52 are rejected under 35 U.S.C. §112, first paragraph as allegedly lacking enablement. Specifically, the Examiner acknowledges that the instant application enables methods of transforming the plastome of a tobacco plant [with a DNA sequence comprising] a mutation at position 226 of SEQ ID NO: 12. *Official Action*, at 3.

In an effort to expedite prosecution, Applicants have defined the claims to encompass tobacco plants only. Consequently the rejection under 35 U.S.C. § 112, first paragraph is now moot. Accordingly, withdrawal of the rejections of claims 28, 29, 34-36, 41, 43 and 52 under 35 U.S.C. § 112, first paragraph is respectfully requested.

Reconsideration of Withdrawn Claims

Claims 42, 44–51, and 53–59 were previously withdrawn but are directed to specific species of independent claims 28 and 35, respectively. If generic claims 28 and 35 are allowed, then the Examiner is respectfully requested to reconsider claims 42, 44–51, and 53–59.

CONCLUSION

All rejections having been addressed and it is respectfully submitted that claims 28, 29, 34–36, and 41–59 are in condition for allowance. A notice to that effect is earnestly solicited. If any points remain in issue, which may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned attorney at the telephone number listed below.

Respectfully submitted,
WOMBLE CARLYLE SANDRIDGE & RICE, PLLC

By: /julie broadus meigs/
Julie Broadus Meigs, Ph.D.
Registration No. 47,447
(703) 394-2253

Date: December 23, 2010

USPTO Customer No. 86805

WCSR 4522166v1

APPENDIX A, continued

301-1	VGSFRGLTUM	LEPAISARIG	DKRVKSWKLS	351-1	351-1	400-1
Rapept-1	VGSFRGLTUM	LEPAISARIG	DKRVKSWKLS	351-1	351-1	400-1
Arabpt-1	VGSFRGLTUM	LEPAISARIG	SVKLSWSKLS	351-1	351-1	400-1
Sorghumpt-1	VASFRGGLAM	LEPAITSISG	SVKLSWSKLS	351-1	351-1	400-1
Mzpt-1	VASFRGGLAM	LEPAITSISG	SVKLSWSKLS	351-1	351-1	400-1
Wtpt-1	VASFRGGLAM	LEPAITSISG	SVKLSWSKLS	351-1	351-1	400-1
Ricept-1	VASFRGGLAM	LEPAITSISG	SVKLSWSKLS	351-1	351-1	400-1
Cotcont-1	VGSFRGLTUM	LEPAINSIG	SVKLSWSKLS	351-1	351-1	400-1
Soybeantpt-1	VGSFRGLTUM	LEPAISARIG	SVKLSWSKLS	351-1	351-1	400-1
Suprt-1	VGSFRGLTUM	LEPAISARIG	SVKLSWSKLS	351-1	351-1	400-1
SEQ ID 12	VGSFRGLTUM	LEPAISARIG	SVKLSWSKLS	351-1	351-1	400-1
AAEL100S222L	VGSFRGLTUM	LEPAISARIG	SVKLSWSKLS	351-1	351-1	400-1
401-1	AIKRECLIDG	ELKGFQGLHP	RQGVETLGT	451-1	451-1	500-1
Rapept-1	AIKRECLIDG	ELKGFQGLHP	RQGVETLGT	451-1	451-1	500-1
Arabpt-1	AIKRECLIDG	ELKGFQGLHP	RQGVETLGT	451-1	451-1	500-1
Sorghumpt-1	AIKRECLIDG	ELKGFQGLHP	RQGVETLGT	451-1	451-1	500-1
Mzpt-1	AIKRECLIDG	ELKGFQGLHP	RQGVETLGT	451-1	451-1	500-1
Wtpt-1	AIKRECLIDG	ELKGFQGLHP	RQGVETLGT	451-1	451-1	500-1
Ricept-1	AIKRECLIDG	ELKGFQGLHP	RQGVETLGT	451-1	451-1	500-1
Cotcont-1	AIKRECLIDG	ELKGFQGLHP	RQGVETLGT	451-1	451-1	500-1
Soybeantpt-1	AIKRECLIDG	ELKGFQGLHP	RQGVETLGT	451-1	451-1	500-1
Suprt-1	AIKRECLIDG	ELKGFQGLHP	RQGVETLGT	451-1	451-1	500-1
SEQ ID 12	AIKRECLIDG	ELKGFQGLHP	RQGVETLGT	451-1	451-1	500-1
AAEL100S222L	AIKRECLIDG	ELKGFQGLHP	RQGVETLGT	451-1	451-1	500-1
501-1	POFLIGHIDL	VDAAKASLUS	SCHEGLFLAG	550-1	550-1	563-1
Rapept-1	POFLIGHIDL	VDAAKASLUS	SCHEGLFLAG	550-1	550-1	563-1
Arabpt-1	POFLIGHIDL	VDAAKASLUS	SCHEGLFLAG	550-1	550-1	563-1
Sorghumpt-1	POFLIGHIDL	VDAAKASLUS	SCHEGLFLAG	550-1	550-1	563-1
Mzpt-1	POFLIGHIDL	VDAAKASLUS	SCHEGLFLAG	550-1	550-1	563-1
Wtpt-1	POFLIGHIDL	VDAAKASLUS	SCHEGLFLAG	550-1	550-1	563-1
Ricept-1	POFLIGHIDL	VDAAKASLUS	SCHEGLFLAG	550-1	550-1	563-1
Cotcont-1	POFLIGHIDL	VDAAKASLUS	SCHEGLFLAG	550-1	550-1	563-1
Soybeantpt-1	POFLIGHIDL	VDAAKASLUS	SCHEGLFLAG	550-1	550-1	563-1
Suprt-1	POFLIGHIDL	VDAAKASLUS	SCHEGLFLAG	550-1	550-1	563-1
SEQ ID 12	POFLIGHIDL	VDAAKASLUS	SCHEGLFLAG	550-1	550-1	563-1
AAEL100S222L	POFLIGHIDL	VDAAKASLUS	SCHEGLFLAG	550-1	550-1	563-1
563-1	POFLIGHIDL	VDAAKASLUS	SCHEGLFLAG	550-1	550-1	563-1
Rapept-1	POFLIGHIDL	VDAAKASLUS	SCHEGLFLAG	550-1	550-1	563-1
Arabpt-1	POFLIGHIDL	VDAAKASLUS	SCHEGLFLAG	550-1	550-1	563-1
Sorghumpt-1	POFLIGHIDL	VDAAKASLUS	SCHEGLFLAG	550-1	550-1	563-1
Mzpt-1	POFLIGHIDL	VDAAKASLUS	SCHEGLFLAG	550-1	550-1	563-1
Wtpt-1	POFLIGHIDL	VDAAKASLUS	SCHEGLFLAG	550-1	550-1	563-1
Ricept-1	POFLIGHIDL	VDAAKASLUS	SCHEGLFLAG	550-1	550-1	563-1
Cotcont-1	POFLIGHIDL	VDAAKASLUS	SCHEGLFLAG	550-1	550-1	563-1
Soybeantpt-1	POFLIGHIDL	VDAAKASLUS	SCHEGLFLAG	550-1	550-1	563-1
Suprt-1	POFLIGHIDL	VDAAKASLUS	SCHEGLFLAG	550-1	550-1	563-1
SEQ ID 12	POFLIGHIDL	VDAAKASLUS	SCHEGLFLAG	550-1	550-1	563-1
AAEL100S222L	POFLIGHIDL	VDAAKASLUS	SCHEGLFLAG	550-1	550-1	563-1

APPENDIX B

US 2005/0081259 A1

Apr. 14, 2005

5

[0074] *Sorghum* protox-1, in the pRfuescript SK vector, was deposited Dec. 6, 1996, as pWDC-19 (NRRL #B-21549).

[0075] Resistant mutant pAraC-2Cys, in the pMut-1 plasmid, was deposited on Nov. 14, 1994 under the designation pWDC-7 with the Agricultural Research Culture Collection and given the deposit designation NRRL #21339N.

[0076] AraPTTPro containing the *Arabidopsis* protox-1 promoter was deposited Dec. 15, 1995, as pWDC-11 (NRRL #B-21515).

[0077] A plasmid containing the maize protox-1 promoter fused to the remainder of the maize protox-1 coding sequence was deposited Mar. 19, 1996 as pWDC-14 (NRRL #B-21546).

[0078] A plasmid containing the sugar beet protox-1 promoter was deposited Dec. 6, 1996, as pWDC-20 (NRRL #B-21550).

Definitions

[0079] For clarity, certain terms used in the specification are defined and explained as follows:

[0080] **Associated With/Operatively Linked:** refers to two DNA sequences that are related physically or functionally. For example, a promoter or regulatory DNA sequence is said to be "associated with" a DNA sequence that codes for an RNA or a protein if the two sequences are operatively linked, or situated such that the regulator DNA sequence will affect the expression level of the coding or structural DNA sequence.

[0081] **Chimeric Gene:** a recombinant DNA sequence in which a promoter or regulatory DNA sequence is operatively linked to, or associated with, a DNA sequence that codes for an mRNA or which is expressed as a protein, such that the regulator DNA sequence is able to regulate transcription or expression of the associated DNA sequence. The regulator DNA sequence of the chimeric gene is not normally operatively linked to the associated DNA sequence as found in nature.

[0082] **Coding DNA Sequence:** a DNA sequence that is translated in an organism to produce a protein.

[0083] **Corresponding To:** in the context of the present invention, "corresponding to" means that when the amino acid sequences of various protox enzymes are aligned with each other, such as in Table 1A, the amino acids that "correspond to" certain enumerated positions in Table 1A are those that align with these positions in Table 1A, but that are not necessarily in these exact numerical positions relative to the particular protox enzyme's amino acid sequence. Likewise, when the amino acid sequence of a particular protox enzyme (for example, the soybean protox enzyme) is aligned with the amino acid sequence of a reference protox enzyme (for example, the *Arabidopsis* protox-1 sequence given in SEQ ID NO.2), the amino acids in the soybean protox sequence that "correspond to" certain enumerated positions of SEQ ID NO.2 are those that align with these positions of SEQ ID NO.2, but are not necessarily in these exact numerical positions of the soybean protox enzyme's amino acid sequence.

[0084] **DNA Shuffling:** DNA shuffling is a method to introduce mutations or rearrangements into a polynucleotide, in a DNA molecule or a method to generate exchanges of DNA sequences between two or more DNA molecules, preferably randomly. The DNA molecule resulting from DNA shuffling is a "shuffled DNA molecule," that is a non-naturally occurring DNA molecule derived from at least one template DNA molecule. The shuffled DNA encodes an enzyme modified with respect to the enzyme encoded by the template DNA, and preferably has an altered biological activity with respect to the enzyme encoded by the template DNA.

[0085] **Herbicide:** a chemical substance used to kill or suppress the growth of plants, plant cells, plant seeds, or plant tissues.

[0086] **Heterologous DNA Sequence:** a DNA sequence not naturally associated with a host cell into which it is introduced, including non-naturally occurring multiple copies of a naturally occurring DNA sequence.

[0087] **Homologous DNA Sequence:** a DNA sequence naturally associated with a host cell into which it is introduced.

[0088] **Homoplasmic:** refers to a plant, plant tissue or plant cell, wherein all of the plastids are genetically identical. In different tissues or stages of development, the plastids may take different forms, e.g., chloroplasts, proplastids, etioplasts, amyloplasts, chromoplasts, and so forth.

[0089] **Inhibitor:** a chemical substance that inactivates the enzymatic activity of a protein such as a biosynthetic enzyme, receptor, signal transduction protein, structural gene product, or transport protein that is essential to the growth or survival of the plant. In the context of the instant invention, an inhibitor is a chemical substance that inactivates the enzymatic activity of protox. The term "herbicide" is used herein to define an inhibitor when applied to plants, plant cells, plant seeds, or plant tissues.

[0090] **Isolated:** in the context of the present invention, an isolated nucleic acid molecule or an isolated enzyme is a nucleic acid molecule or enzyme that, by the hand of man, exists apart from its native environment and is therefore not a product of nature. An isolated nucleic acid molecule or enzyme may exist in a purified form or may exist in a non-native environment such as, for example, a transgenic host cell.

[0091] **Minimal Promoter:** promoter elements, particularly a TATA element, that are inactive or that have greatly reduced promoter activity in the absence of upstream activation. In the presence of a suitable transcription factor, the minimal promoter functions to permit transcription.

[0092] **Modified Enzyme Activity:** enzyme activity different from that which naturally occurs in a plant (i.e. enzyme activity that occurs naturally in the absence of direct or indirect manipulation of such activity by man), which is tolerant to inhibitors that inhibit the naturally occurring enzyme activity.

[0093] **Nucleic Acid Molecule:** a linear segment of single- or double-stranded DNA or RNA that can be isolated from any source. In the context of the present invention, the nucleic acid molecule is preferably a segment of DNA.